lently cross-linked, aggregates. The core proteins are first deposited by the cell and, as a function of time in culture, fibronectin gradually coats their surface.

Schwartz, E., *Goldfischer, S.*, Coltoff-Schiller, B., and Blumenfeld, O. O.

The Journal of Histochemistry and Cytochemistry 33(4):268-274, 1985.

Other support: National Institutes of Health and the David Opochinsky-Henry Segal and the Blumenfeld Family Memorial Funds.

From the Departments of Biochemistry and Pathology, Albert Einstein College of Medicine, The Bronx, NY.

IMMUNOLOGIC INHIBITION OF ULTRAVIOLET RADIATION-INDUCED TUMOR SUPPRESSOR CELL ACTIVITY.

Long-term exposure of C3H mice to ultraviolet radiation resulted in the formation of suppressor T cells that recognize ultraviolet radiation-induced regressor skin cancers as a class before the appearance of overt tumors. Administration of monoclonal antibodies to the product of the I-J^k subregion of the major histocompatibility complex or low doses of cyclophosphamide in vivo inhibited the development or activity of these cells. This activity of the monoclonal antibody was eliminated by adsorption on B10.BR (I-J^k) but not B10.D2 (I-J^d) splenocytes. These findings provide evidence that elements expressing the I-J determinant are important in regulating the host response prior to the overt development of ultraviolet radiation-induced skin cancers and suggest novel therapeutic approaches to malignancies or other diseases involving suppressor T cells in their pathogenesis.

Granstein, R. D., Parrish, J. A., McAuliffe, D. J., Waltenbaugh, C., and *Greene, M. I.*

Science 224:615-617, 1984.

Other support: National Research Service Award and Arthur O. and Gullan M. Wellman Foundation.

From the Departments of Dermatology and Pathology, Harvard Medical School and Massachusetts General Hospital, Boston, and Department of Microbiology and Immunology, Northwestern University Medical School, Chicago.

EPIDERMAL ANTIGEN-PRESENTING CELLS IN ACTIVATION OF SUPPRESSION: IDENTIFICATION OF A NEW FUNCTIONAL TYPE OF ULTRAVIOLET RADIATION-RESISTANT EPIDERMAL CELL

In recent years, evidence has accumulated for the presence of immunologically active elements resident in the skin, which has led to the concept of skin-associated lymphoid tissues (SALT). Immunologic functions of these elements have been demonstrated to include processing and presentation of antigen to lymphocytes by dendritic cells called epidermal Langerhans cells (LC). Additionally, epidermal cell-derived, thymocyte-activating factor (ETAF), an entity biochemically and functionally very similar or identical to interleukin 1 (IL1), is produced by keratinocytes. Thus, the skin can be considered a complex organ in which cells relevant to the immune system are represented

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Exposure of mice to low doses of ultraviolet radiation (UVR) *in vivo* leads to an inability to sensitize them to contact sensitizing reagents at the site of irradiation, and this depressed sensitization is accompanied by the formation of antigen-specific T suppressor (Ts) cells. It has also been demonstrated that hapten-coupled epidermal cells (EC) from UVR-treated mice are unable to immunize mice efficiently when administered subcutaneously, and such immunization results in the appearance of suppressor cells. We report that the murine epidermis contains a previously unrecognized antigen-presenting cell (APC) that is required for the activation of suppression, and that this APC is resistant to UVR. The dose of UVR employed is, however, sufficient to prevent substantial positive immunization of mice with syngeneic UV-irradiated hapten-coupled EC. These data explain in part the changes induced in epidermal antigen-presenting function by UVR, and has consequences for the understanding of UVR-induced cutaneous carcinogenesis.

Granstein, R. D., Lowy, A., and *Greene, M. I.*

The Journal of Immunology 132(2):563-565, 1985.

Other support: National Eye Institute, National Institutes of Health and Arthur O. and Gullan M. Wellman Foundation.

From the Departments of Dermatology and Pathology, Harvard Medical School, and Massachusetts General Hospital, Boston.

SPLENIC I-J BEARING ANTIGEN-PRESENTING CELLS IN ACTIVATION OF SUPPRESSION: FURTHER CHARACTERIZATION

A set of I-J-bearing murine splenic antigen-presenting cells (APC) has been found to be responsible for first order suppressor cell (Ts1, afferent suppressor cell) activation in the azobenzenearsonate (ABA) hapten system after intravenous administration. Suppressor cells induced by this set of hapten-coupled cells do not function in the efferent phase of the delayed hypersensitivity (DTH) response. The functional activity of this novel APC to activate afferent suppressor cells was resistant to a dose of ultraviolet radiation (UVR) sufficient to largely abrogate the ability of splenic APC to immunize for a DTH response. It was also found that the previously described splenic I-J-bearing APC needed for third-order suppressor cell (Ts3, effector-suppressor cell) activation is adherent and UVR resistant. The sets of I-J-bearing APC appear to be crucial elements in the activation of suppression and thus in determining the balance between immunologic reactivity and unresponsiveness. Furthermore, the UVR resistance of this set of novel APC may be relevant to the *in vivo* effects of UVR exposure to mice.

Granstein, R. D. and *Greene, M. I.*

Cellular Immunology 91:12-20, 1985.

Other support: Arthur O. and Gullan M. Wellman Foundation.

From the Departments of Dermatology and Pathology, Harvard Medical School and Massachusetts General Hospital, Boston.

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MONOCLONAL IDIOTOPE VACCINE AGAINST *STREPTOCOCCUS PNEUMONIAE* INFECTION

A monoclonal anti-idiotope antibody coupled to a carrier protein was used to immunize BALB/c mice against a lethal *Streptococcus pneumoniae* infection. Vaccinated mice developed a high titer of antibody to phosphorylcholine, which is known to protect against infection with *Streptococcus pneumoniae*. Measurement of the median lethal dose of the bacteria indicated that anti-idiotope immunization significantly increased the resistance of BALB/c mice to the bacterial challenge. Antibody to an idiotope can thus be used as an antigen substitute for the induction of protective immunity.

McNamara, M. K., Ward, R. E. and *Kohler, H.*

Science 226:1325-1326, 1984.

Other support: U.S. Public Health Service and the New York State Department of Health AIDS Institute.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

ANALYSIS OF A $T_{H1} \rightarrow T_{H2}$ HELPER CELL CIRCUIT

In the present study, the T15 idiotype-recognizing T helper cell circuit was dissected with respect to its homeostasis, interactive specificity, stability over time and effects on B cell expression. Analysis of the T_{H1} cells by adoptive transfer experiments indicates their short-lived state of activity during which T_{H2} cells are stimulated. T_{H1} cell activity was also directly monitored by the use of TNP-anti-T15 hybridoma antigens. It was found that T_{H1} cells are detected 1 wk after priming with PC-Hy, whereas T_{H2} cells become activated after 4 wk of priming. Comparative analysis of T_{H1} cells by using two different TNP-anti-T15 hybridoma antigens indicates a T_{H1} specificity for a shared idiotope. The stability over time of the $T_{H1} \rightarrow T_{H2}$ circuit was demonstrated by comparing T_{H2} frequencies in young and old mice.

Finally, we addressed the question of the function of the idiotype-recognizing T helper cells and showed that stimulation of T15-idiotype-specific T_{H2} cells can be correlated with a significant increase in the percentage of T15 idiotype in an anti-PC response. Collectively, these data describe an idiotype-specific T helper circuit as part of the network homeostasis of the immune system.

McNamara, M., Kang, C.-Y., and *Kohler, H.*

The Journal of Immunology 135(3):1603-1609, 1985.

Other support: National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

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IMMUNOREACTIVE CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P COEXIST IN SENSORY NEURONS TO THE SPINAL CORD AND INTERACT IN SPINAL BEHAVIORAL RESPONSES OF THE RAT

Using immunohistochemistry, evidence was obtained for the coexistence of calcitonin gene-related peptide (CGRP)- and substance P (SP)-like immunoreactivity in spinal sensory neurons. Analysis of caudally directed biting and scratching (CBS) behavior was carried out after intrathecal administration of CGRP and SP alone or in combination. Thus, SP (up to 20 μg) alone caused CBS only for a few minutes after injection, whereas SP (10 μg) plus CGRP (20 μg) caused a response with a duration up to 40 min. CGRP (20 μg) alone had no effects in this model. These findings provide support for a possible interaction of the two peptides at synapses in the dorsal horn of the spinal cord.

Wiesenfeld-Hallin, Z., Hokfelt, T., *Lundberg, J. M.*, Forssmann, W. G., Reinecke, M., Tshopp, F. A., and Fischer, J. A.

Neuroscience Letters 52:199-204, 1984.

Other support: The Swedish Medical Research Council, the Folksam Insurance Company, the Karolinska Institute, and the Swiss National Science Foundation.

From the Department of Clinical Neurophysiology, Huddinge Hospital; Departments of Histology and Pharmacology, Karolinska Institute, Stockholm; Department of Anatomy III, University of Heidelberg, Federal Republic of Germany; and the Research Laboratory for Calcium Metabolism, Departments of Orthopedic Surgery and Medicine, University of Zurich, Switzerland.

BIOSYNTHESIS OF GLYCOSPHINGOLIPIDS BY HUMAN MYELOID LEUKEMIA CELLS

We have performed comparative studies of the neutral glycosphingolipids synthesized by three human myeloid leukemia cell lines, K562, KG1, and HL-60, which were metabolically labeled with [^{14}C]galactose, to evaluate changes in neutral glycosphingolipid synthesis with myeloid cell differentiation. Individual neutral glycosphingolipids containing one to four sugars were purified by a combination of the following methods: diethylaminoethyl-Sephadex column chromatography, acetylation-Florisil column chromatography, and high-performance liquid chromatography using an Iatrobead column. Compounds with one sugar were analyzed by thin-layer chromatography on borate plates. This analysis showed that HL-60 cells synthesize only glucosylceramide, whereas K562 and KG1 cells synthesize predominantly [illegible]osylceramide, but also a small amount of galactosylceramide. Compounds with two to four s[illegible] were characterized by treatment with exo- and endoglycosidases. The results showed that K562 and KG1 cells are similar to cells from patients with acute leukemia in expressing two series (globo and neolacto) of natural glycosphingolipids, whereas the HL-60 cells are similar to mature human myeloid cells in expressing only one series (neolacto). Therefore, human myeloid leukemia cells blocked at different stages of differentiation vary in their ability to synthesize neutral glycosphingolipids.

Buehler, J., Qwan, E., DeGregoria, M. W., and *Macher, B. A.*

Biochemistry 24:6978-6984, 1985.

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Other support: The Louis R. Lurie Foundation and the National Cancer Institute.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco; Children's Cancer Research Institute, Pacific Presbyterian Medical Center, San Francisco.

HUMAN NATURAL ANTI-α-GALACTOSYL IgG II. THE SPECIFIC RECOGNITION OF α(1 →)-LINKED GALACTOSE RESIDUES

A natural IgG antibody (anti-Gal) with α-galactosyl binding specificity has been found in large amounts (0.5-1.0% of serum IgG) in all individuals tested. It has been purified by affinity chromatography on a column of melibiose-Sepharose. In addition to its affinity for normal and pathological senescent human red cells, the antibody readily interacts with rabbit red blood cell (RRBC) glycolipids with α-galactosyl terminal residues. Two types (glycosidic linkages of 1 → 3 *vs.* 1 → 4) of rabbit red cells glycolipids with terminal α-galactosyl residues were tested for antibody binding. The antibody specifically bound to glycolipids with Galα1 → 3 terminal residues, and treatment of these glycolipids with α-galactosidase abolished binding. Hemagglutination inhibition studies with oligosaccharides of known structure also showed that the antibody binds specifically to glycoconjugates with an α1 → 3 terminal galactose residue. Anti Gal did not bind to a human B-active glycolipid, indicating that fucose-linked α1 → 2 to the penultimate galactose prevents anti-Gal binding. The anti-Gal specificity for RRBC glycolipids also paralleled that of the α-galactosyl-specific *Bandeiraea simplicifolia* lectin. The possible reasons for the occurrence of this unique antibody in human serum are discussed.

Galili, U., *Macher, B. A.*, Buehler, J., and Shohet, S. B.

Journal of Experimental Medicine 162:573-582, 1985.

Other Support: National Institutes of Health.

From the MacMillan-Cargill Hematology Research Laboratory, Cancer Research Institute, Departments of Pharmaceutical Chemistry and Laboratory Medicine, University of California, San Francisco.

GLYCOSPHINGOLIPID CARRIERS OF CARBOHYDRATE ANTIGENS OF HUMAN MYELOID CELLS RECOGNIZED BY MONOCLONAL ANTIBODIES

Six monoclonal antibodies with known specificities for the carbohydrate antigens i, X or Y, and several anti-myeloid antibodies (determinants unknown) selected for their differing reaction patterns with human leucocytes were tested in chromatogram binding assays for reactions with myeloid cell glycolipids derived from normal human granulocytes and chronic myelogenous leukemia cells. Antigenicities were found exclusively on minor glycolipids which were barely or not at all detectable with orcinol-sulphuric acid stain. Among these, a neutral glycosphingolipid bound the anti-i antibody Den and chromatographed as the ceramide octasaccharide, (Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc-Cer. Several species of neutral glycosphingolipids with six to more than ten monosaccharides were detected that carry the X antigen and others the Y antigen: Galβ1 → 4(Fucα1 → 3)GlcNAc and Fucα1 → 2Galβ1 → 4(Fucα1 → 3)GlcNAc, respectively. In addition, three new types of

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carbohydrate specificities were detected among the myeloid cell glycolipids. Two were associated with neutral glycolipids: the first, recognized by anti-myeloid antibodies VIM-1 and VIM-10, was expressed on a distinct set of glycolipids with six or more monosaccharides, and the second, recognized by VIM-8, was expressed on glycolipids with more than ten monosaccharides. The third specificity, recognized by the anti-myeloid antibody VIM-2, was expressed on slow migrating sialoglycolipids with backbone structures of the poly-N-acetyllactosamine type that are susceptible to degradation with endo-β-galacosidase. Thus, we conclude that the i and Y antigens occur among the glycolipids of normal myeloid and chronic myelogenous leukemia cells and that a high proportion of hybridoma antibodies raised against differentiation antigens of myeloid cells are directed at carbohydrate structures.

Uemura, K., *Macher, B. A.*, DeGregorio, M., Scudder, P., Buehler, J., Knapp, W., and Feizi, T.

Biochimica et Biophysica Acta 846:26-36, 1985.

Other support: National Cancer Institute, North Atlantic Treaty Organization and Louis R. Lurie Foundation.

From the Applied Immunochemistry Research Group, Division of Communicable Diseases; Medical Research Council's Clinical Research Center, Middlesex (U.K.); Cancer Research Institute, Department of Pharmaceutical Chemistry, University of California, San Francisco; Children's Cancer Research Institute; Pacific Medical Center; and Department of Internal Medicine, Institute of Immunology, University of Vienna, Austria.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA* I. EVIDENCE THAT A LYMPHOKINE MEDIATES KILLING

Previous studies have demonstrated *in vivo* that T cells can provide protective immunity, in the absence of antibody, against infection with the extracellular Gram-negative bacterium Immunotype 1 (IT-1) *Pseudomonas aeruginosa*. We established an *in vitro* system in which immune T cells, after reexposure to bacterial antigens and to macrophages, secrete a product that kills the bacteria. Although macrophages are required for *in vitro* killing, they function neither as antigen-presenting nor as phagocytic cells in this system. T cells from animals immunized against a different *P. aeruginosa* immunotype will not kill IT-1 organisms, but the supernatants produced by IT-1 immune T cells after exposure to macrophages and IT-1 *P. aeruginosa* organisms are nonspecifically effective in killing unrelated bacteria. Because the supernatants from immune T cells lose their bactericidal properties upon minimal dilution, we conclude that if this mechanism is active *in vivo*, it must play a role in local immunity.

Markham, R. B., Goellner, J., and Pier, G. B.

The Journal of Immunology 133(2):962-968, 1984.

Other support: Auxiliary of the Jewish Hospital of St. Louis; U.S. Public Health Service; National Institutes of Health; and U. S. Army Research and Development Command.

From the Departments of Medicine, and Microbiology & Immunology, Washington University School of Medicine, and The Jewish Hospital of St. Louis; Channing Laboratory, Harvard Medical School, and Brigham and Women's Hospital, Boston.

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MEMBRANE AND CYTOSKELETAL CHANGES ASSOCIATED WITH IgE-MEDIATED SEROTONIN RELEASE FROM RAT BASOPHILIC LEUKEMIA CELLS

Binding of antigen to IgE-receptor complexes on the surface of RBL-2H3 rat basophilic leukemia cells is the first event leading to the release of cellular serotonin, histamine and other mediators of allergic, asthmatic and inflammatory responses. We have used dinitrophenol-conjugated bovine serum albumin (DNP-BSA) as well as the fluorescent antigen, DNP-B-phycoerythrin, and the electron-dense antigen, DNP-BSA-gold, to investigate dynamic membrane and cytoskeletal events associated with the release of [^{3}H]serotonin from anti-DNP-IgE-primed RBL-2H3 cells. These multivalent antigens bind rapidly to cell surface IgE-receptor complexes. Their distribution is initially uniform, but within 2 min DNP-BSA-gold is found in coated pits and is subsequently internalized. Antigen internalization occurs in the presence and absence of extracellular Ca^{2+}. The F-actin content of the detergent-extracted cell matrices analyzed by SDS PAGE decreases during the first 10–30 s of antigen binding and then increases by 1 min to almost double the control levels. A rapid and sustained increase is also observed when total F-actin is quantified by flow cytometry after binding of rhodamine-phalloidin. The antigen-stimulated increase in F-actin coincides with (and may cause) the transformation of the cell surface from a finely microvillous to a highly folded or plicated topography. Other early membrane responses include increased cell spreading and a 2-3-fold increase in the uptake of fluorescein-dextran by fluid pinocytosis. The surface and F-actin changes show the same dependence on DNP-protein concentration as stimulated [^{3}H] serotonin release; and both the membrane responses and the release of mediators are terminated by the addition of the non-cross-linking monovalent ligand, DNP-lysine. These data indicate that the same antigen-stimulated transduction pathway controls both the membrane/cytoskeletal and secretory events. However, the membrane and actin responses to IgE-receptor-cross-linking are independent of extracellular Ca^{2+} and are mimicked by phorbol myristate acetate, whereas ligand-dependent mediator release depends on extracellular Ca^{2+} and is mimicked by the Ca^{2+} ionophore A23187.

Pfeiffer, J. R., Seagrave, J. C., Davis, B. H., Deanin, G. D., and *Oliver, J. M.*

The Journal of Cell Biology 101:2145-2155, 1985.

Other support: American Cancer Society and National Institutes of Health.

From the Department of Pathology, University of New Mexico School of Medicine, Albuquerque, and the Department of Pathology, State University of New York, Upstate Medical Center, Syracuse.

DISTRIBUTION OF GALANIN IMMUNOREACTIVITY IN THE RESPIRATORY TRACT OF PIG, GUINEA PIG, RAT, AND DOG

Galanin, a newly discovered peptide isolated from porcine intestine, is known to cause contraction in rat smooth muscle preparations and to induce hyperglycemia in dogs. By the use of radioimmunoassay and immunohistochemical techniques the concentration and distribution of galanin immunoreactivity were determined in several areas of the respiratory tract of five dogs, five guinea pigs, five rats, and two pigs. Antibodies were raised in rabbits to whole unconjugated natural porcine galanin. The highest galanin concentrations were found in the bronchus and the trachea of the dog,

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guinea pig, rat (2 pmol/g in each case), and pig (≤ 1 pmol/g). The lowest galanin concentrations were found in the lung parenchyma. Gel chromatographic analysis in the pig showed one molecular form of galanin coeluting with the porcine galanin standard. By means of the indirect immunofluorescence technique on sections of tissues fixed in benzoquinone solution, galanin was found to be confined to nerve fibres in different regions of the respiratory tract. In the nasal mucosa of the pig, nerve fibers containing galanin were distributed around seromucus glands and blood vessels and beneath the epithelium. In the trachea, bronchus, and major intrapulmonary airway of the pig, dog, and guinea pig, galanin immunoreactive fibers were detected predominantly in smooth muscle, as well as around seromucus glands and in the adventitia of blood vessels. Rarely, galanin immunoreactive nerve fibers were found in the lung parenchyma. A few galanin immunoreactive ganglion cells also containing vasoactive intestinal polypeptide were found in the adventititia of the tracheobronchial wall of the pig and dog. The distribution of galanin suggests that this peptide may have some influence on airway, vascular and secretory functions in the mammalian respiratory tract.

Cheung, A., *Polak, J. M.*, Bauer, F. E., Cadiux, A, Christofides, N. D., Springall, D. R., and Bloom, S. R.

Thorax 40:889-896, 1985.

Other support: Medical Research Council (United Kingdom).

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, London, England.

ANALYSIS OF MACROPHAGE DIFFERENTIATION AND FUNCTION WITH MONOCLONAL ANTIBODIES

A large number of anti-mouse and anti-human macrophage/monocyte monoclonal antibodies (MAb) have recently been obtained that are proving invaluable reagents of extraordinary specificity for the study of macrophage differentiation, function, and surface antigen structure. This chapter summarizes information on such MAb up to February 1983. In the mouse, at least five antigens that can be distinguished by molecular weight have been found on macrophages but not on lymphocytes. One of the mouse Fc receptors, present on macrophages as well as on some lymphocytes, has also been defined with MAb. Further antigens have not been defined biochemically but appear to have distinct distributions on functional subpopulations. Mac-1 was the first antigen to be defined by MAb that is present on macrophages and not on lymphocytes. A second antigen has been discovered that is distinct from Mac-1 in cell distribution, function, and α-subunit structure, but appears to use the same β-subunit. In the course of studies on the molecular basis of T-cell function, MAb were selected for their ability to inhibit antigen-specific T-lymphocyte-mediated killing. It appears that LFA-1 is distinct from the antigen receptor, but works together with it in contributing to the avidity of the CTL for the target cell. LFA-1 is present on B lymphocytes and myeloid cells as well as T lymphocytes, suggesting that it plays a more general role in adhesion than do antigen receptors. A similar family of related molecules has been found on human cells. Other sections of this study deal with MAC-2 antigen, Mac-3 antigen, Langerhans cells, dendritic cells, and macrophages, defining macrophages by their surface markers, and immunological evolution.

Springer, T. A. and Unkeless, J. D.

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

In: Adams, D. O. and Hanna, M. G., Jr. (eds.): *Contemporary Topics in Immunobiology* Vol. 13, New York: Plenum Publishing Corporation, 1984, pp. 1-31.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Laboratory of Membrane Immunochemistry, Harvard Medicall School, Boston, and the Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York.

PREPARATION AND USE OF MONOCLONAL ANTIMACROPHAGE ANTIBODIES

Macrophages are a diverse family of cells originating from pluripotent stem cells in the bone marrow. Outside the marrow, macrophages can take the form of blood monocytes, Kupffer cells, alveolar macrophages, peritoneal macrophages, Langerhans cells, and in almost every organ, as "fixed tissue macrophages." In addition to variation in anatomical localizations, macrophages can exhibit heterogeneity in function and state of differentiation. The advent of hybridoma technology has allowed the identification of over 40 macrophage antigens, some of which are useful for distinguishing macrophages from other cells whereas others are associated with distinct subsets of macrophages. In this section, a number of monoclonal antibodies with relatively restricted specificities for macrophages were chosen and their characteristics were summarized. Possible applications of these antibodies are also described.

Ho, M.-K. and *Springer, T. A.*

Methods in Enzymology 108:313-325, 1984.

Other support: U. S. Public Health Service.

From Harvard Medical School, Boston.

THE LFA-1, Mac-1 GLYCOPROTEIN FAMILY AND ITS DEFICIENCY IN AN INHERITED DISEASE

A family of functionally important, high-molecular-weight glycoproteins with identical β subunits has recently been defined on leukocyte cell surfaces. Soon after these molecules and at least some of their functions had been defined with monoclonal antibodies, an inherited disease, LFA-1, Mac-1 deficiency, was discovered in humans. This deficiency has confirmed that this glycoprotein family is of central importance in leukocyte cell surface adhesion reactions.

Springer, T. A.

Federation Proceedings 44:2660-2663, 1985.

Other support: National Institutes of Health.

From the Dana-Farber Cancer Institute, Harvard Medical School, Boston.

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THE SEVERE AND MODERATE PHENOTYPES OF HERITABLE Mac-1, LFA-L DEFICIENCY: THEIR QUANTITATIVE DEFINITION AND RELATION TO LEUKOCYTE DYSFUNCTION AND CLINICAL FEATURES.

An inherited syndrome characterized by recurrent or progressive necrotic soft-tissue infections, diminished pus formation, impaired wound healing, granulocytosis, and/or delayed umbilical cord severance was recognized in four male and four female patients. As shown with subunit-specific monoclonal antibodies in immunofluorescence flow cytometry and ^{125}I immunoprecipitation techniques, in addition to a tritiated sodium borohydride-galactose oxidase labeling assay, granulocytes, monocytes or lymphocytes from these individuals had a "moderate" or "severe" deficiency of Mac-1, LFA-1, or p150,95 (or a combination) - three structurally related "adhesive" surface glycoproteins. Two distinct phenotypes were defined on the basis of the quantity of antigen expressed. Three patients with severe deficiency and four patients with moderate deficiency expressed < 0.3% and 2.5-31% of normal amounts of these molecules on granulocyte surfaces, respectively. The severity of clinical infectious complications among these patients was directly related to the degree of glycoprotein deficiency. More profound abnormalities of tissue leukocyte mobilization, granulocyte-directed migration, hyperadherence, phagocytosis of iC3b-opsonized particles, and complement- or antibody-dependent cytotoxicity were found in individuals with severe, as compared with moderate, deficiency. It is proposed that *in vivo* abnormalities of leukocyte mobilization reflect the critical roles of Mac-1 glycoproteins in adhesive events required for endothelial margination and tissue exudation. The recognition of phenotypic variation among patients with Mac-1, LFA-1 deficiency may be important with respect to therapeutic strategies.

Anderson, D. C. *Springer, T. A. et al.*

The Journal of Infectious Diseases 152(4):668-689, 1985.

Other support: National Institute of Allergy and Infectious Diseases, National Institute for Cancer Research, National Institutes of Health, and U. S. Department of Agriculture.

From the Departments of Pediatrics, Microbiology and Immunology, and Pathology, Baylor College of Medicine, Houston, and Dana-Farber Cancer Institute, Harvard Medical School, Boston.

MACROPHAGE AND T LYMPHOCYTE-MEDIATED IMMUNITY: SIMILARITIES AT THE LEVEL OF THE MAC-1 AND LFA-1 MOLECULES

A number of cell surface molecules have recently been characterized which are important in macrophage and lymphocyte cell-mediated immunity. The macrophage Fc receptor and complement receptor type one (CR_1) have been characterized by purification and function-inhibiting antibodies. Recently, anti-Mac-1 antibody was found to inhibit the complement receptor type three (CR_3), suggesting that the CR_3 is identical to or associated with the previously biochemically characterized Mac-1 molecule. Studies on T lymphocyte-mediated immunity with function-blocking antibodies have shown that Lyt-2,3 and LFA-1 surface molecules are associated with T lymphocyte-mediated killing. Somewhat surprisingly, the Mac-1 molecule associated with CR_1 function, and the LFA-1 molecule associated with T-lymphocyte-mediated killing, have been found to be structurally related. This suggests that at least with regard

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to these molecules, similar molecular mechanisms underlie macrophage and T lymphocyte-mediated immunity. The findings on Mac-1 and LFA-1 are reviewed and the evolutionary implications are discussed.

Springer, T. A.

In: Volkman, A. (ed.): *Mononuclear Phagocyte Biology*, New York: Marcel Dekker, 1984, pp. 109-128.

Other support: U. S. Public Health Service and an American Cancer Society Junior Faculty Award.

From Harvard Medical School, Boston.

FUNCTIONAL AND STRUCTURAL INTERRELATIONSHIPS AMONG THE Mac-1, LFA-1 FAMILY OF LEUKOCYTE ADHESION GLYCOPROTEINS, AND THEIR DEFICIENCY IN A NOVEL HERITABLE DISEASE

Cell surface adherence reactions are of central importance in the immune functions of lymphocytes, monocytes, and granulocytes. Lymphocytes adhere to antigen-presenting macrophages or dendritic cells in the induction of T-lymphocyte immune responses and to target cells in cell-mediated killing. Adhesive interactions are fundamental to a wide spectrum of functions of granulocytes, monocytes, and macrophages. Specific recognition of opsonized microorganisms is facilitated by membrane receptors for IgG and for the third component of complement (C3), which mediate microbe-cell adhesion prior to the triggering of cytoskeletal events leading to endocytosis. Adhesion mediated by IgG (Fc) receptors can also trigger antibody-dependent killing of target cells, independently of endocytosis. A family of high-molecular-weight glycoproteins with identical β subunits has recently been characterized on leukocyte surfaces that is important in many of the above adhesion reactions. Monoclonal antibodies have been instrumental in elucidating the structural interrelationships and functions of these three glycoproteins, macrophage antigen 1 (Mac-1), lymphocyte function-associated antigen 1 (LFA-1), and p150,95. Furthermore, this research has led to the definition, with monoclonal antibodies, of a novel heritable disease that manifests itself in defects in leukocyte adherence and motility. With the use of MAb, a novel disease has been recognized in which the Mac-1, LFA-1 and p150,95 glycoproteins are deficient. Recurrent bacterial infection, progressive periodontitis, persistent leukocytosis, and/or delayed umbilical cord separation have been described in patients whose neutrophils demonstrated depressed phagocytic function and deficient adherence and chemotaxis.

Springer, T. A. and Anderson, D. C.

In: Springer, T. A. (ed.): *Hybridoma Technology in the Biosciences and Medicine*, Chap. 11, New York: Plenum Publishing Coporation, 1985, pp. 101-206.

Other support: National Institutes of Health.

From the Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, and Baylor College of Medicine, Department of Pediatrics, Houston, TX.

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STUDIES ON ANTIGENS ASSOCIATED WITH THE ACTIVATION OF MURINE MONONUCLEAR PHAGOCYTES: KINETICS OF AND REQUIREMENTS FOR INDUCTION OF LYMPHOCYTE FUNCTION-ASSOCIATED (LFA)-1 ANTIGEN *IN VITRO*

Macrophages activated and primed *in vivo*, although not resident or responsive macrophages, express the lymphocyte function associated (LFA)-1 antigen. By contrast, the biochemically related Mac-1 antigen is expressed on all populations of macrophages. In the present paper, we studied regulation of the LFA-1 antigen in vitro. LFA-1 could be induced *in vitro* on thioglycollate (TG)-elicited but not on proteose peptone (PP)-elicited or resident macrophages. Specifically, macrophage-activating factor (MAF), interferon-γ (IFN-γ), or picogram amounts of endotoxin (LPS) induced LFA-1 on TG-elicited macrophages following overnight incubation. Interferon, -α or -β, fucoidin, and colony-stimulating factor were not effective. While some levels of LFA-1 could be detected as soon as 10 hr peak expression was observed after 16 to 32 hr of incubation. The induction could be completely abrogated by cycloheximide, suggesting that protein synthesis was required. These results indicate that the induction of LFA-1 on mononuclear phagocytes is closely regulated and that the requirements for such induction are distinct from, but share certain similarities with, induction of cytotoxic functions and expression of Ia antigen

Strassmann, G., *Springer, T. A.*, and Adams, D. O.

The Journal of Immunology 135(1):17-150, 1985.

From the Department of Pathology, Duke University Medical Center, Durham, NC, and the Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Harvard Medical School, Boston.

COMPLEMENT RECEPTOR TYPE THREE-DEPENDENT DEGRADATION OF OPSONIZED ERYTHROCYTES BY MOUSE MACROPHAGES

The role of the complement receptor type 3 (CR3) on thioglycollate-elicited peritoneal macrophages (TG-PM) in the destruction of opsonized particles was studied. We found that sheep red blood cells (E) that were opsonized with an IgM monoclonal anti-Forssman antibody and complement (E-IgM-C) were lysed by TG-PM, whereas there was little lysis of E pretreated with either the antibody or the complement source alone. Furthermore, this lysis could be inhibited by anti-CR3 monoclonal antibodies that had previously been shown to inhibit binding of E-IgM-C to the CR3. Kinetic studies of phagocytosis and lysis indicated that lysis of E-IgM-C occurs after phagocytosis, suggesting that lysis is an intracellular event. Further findings suggested that intracellular lysis was promoted by CR3 bound to the phagocytosed target, because a monoclonal anti-CR3 antibody decreased the rate of phagocytosis of E-IgM-C but not its magnitude, whereas the rate and extent of lysis were strikingly inhibited. Furthermore, TG-PM that had already internalized unopsonized E selectively lysed E-IgM-C that were added later. These data confirm that the interaction of the CR3 with its ligand on E-IgM-C promotes rapid phagocytosis, and they further suggest that the CR3 facilitates degradation of the target particle once internalization has occurred.

Rothlein, R. and *Springer, T. A.*

The Journal of Immunology 135(4):2268-2672, 1985.

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Other support: U.S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Harvard Medical School, Boston.

THE FUNCTION OF LFA-1 IN CELL-MEDIATED KILLING AND ADHESION: STUDIES ON HERITABLE LFA-1, Mac-1 DEFICIENCY AND ON LYMPHOID CELL SELF-AGGREGATION

Lymphocyte function associated antigen-1 (LFA-1) is a cell surface glycoprotein identified in mouse and human by monoclonal antibodies which inhibit cytolytic T lymphocyte (CTL) mediated cytolysis. Recently, a number of patients with recurring, life-threatening infections were found to be deficient in LFA-1 and two other surface molecules which utilize the same β subunit, Mac-1 and p150,95. To assess natural killing, peripheral blood lymphocytes (PBL) from LFA-1 deficient individuals, their families, and unrelated controls were cultured alone or with JY cells for six days. Natural killing was assessed on the K562 erythroleukemia cell line (HLA negative). All LFA-1 deficient individuals showed low levels of NK cell-mediated cytolysis compared to that of family members and unrelated individuals. PBL were also tested for proliferation after stimulation with the lectin phytohemagglutinin (PHA). PBL from LFA-1 deficient patients showed an impaired proliferative response to PHA. Contrasting results were found for the quantitatively more severely LFA-1 deficient CTL of patients 1 and 2. Killing by patient 2 CTL was poorly inhibited when anti-LFA-1 MAb was added to the assay, and pretreatment of killers or effectors gave equivical or no blocking. CTL from patient 1 was inhibited when anti-LFA-1 was included in the assay. The findings with patient 1 and 4 CTL suggest that LFA-1 on target cells can also contribute to the CTL-target interaction. Data presented here suggest a strong simularity between phorbol ester-induced cell-cell aggregation and the LFA-1-dependent adhesion step in CTL-mediated killing, and suggest LFA-1 functions as an adhesion protein.

Springer, T. A. et al.

In: Henlart, P. and Martz, E. (eds.): *Mechanisms of Cell-Mediated Cytotoxicity II*. New York & London: Plenum Press, 1985, pp. 311-322.

Other support: National Institutes of Health.

From Dana-Farber Cancer Institute, Harvard Medical School, Boston; Baylor College of Medicine, Houston, TX; and Stanford University, Stanford, CA.

STRUCTURAL ORGANIZATION OF INTERPHASE 3T3 FIBROBLASTS STUDIED BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

We studied the laminar organization of 3T3 fibroblast cells growing on glass slides by use of total internal reflection illumination to excite fluorescence emission (TIRF) from labeled molecules and stained cellular compartments that are very close to the cell-substrate contact region. Mitochondria, distant from the contact regions and stained with the water-soluble cationic dye, dil-C_3-(3), fluoresced only as the glass/cytoplasm critical angle was approached. A similar result was obtained when the nuclei were stained with Hoechst dye 33342. From this measured angle a cytoplasmic refractive index in the range 1.358-1.374 was computed. The plasma membrane of 3T3 cells was stained with dil-C_{18}-(3) and the cytoplasmic compartment was stained with fluoresceinyl-dextran (FTC dextran) or with carboxyfluorescein. We have

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demonstrated a high degree of correspondence between the low-reflectance zones in the reflection interference image of a live cell and the TIRF images of both the plasma membrane and cytoplasmic compartment. TIRF photometry of selected contact regions of cells provided data from which the absolute separation of cell and substrate was computed. From a population of 3T3 cells microinjected with fluorescein-labeled actin, motile and adherent interphase cells were selected for study. For adherent cells, which displayed fluorescent stress fibers, the TIRF image was composed of intense patches and less intense regions that corresponded, respectively, to the focal contact and close-contact zones of the reflection-interference image. The intense patches corresponded to the endpoints of the stress fibers. Cells of motile morphology, which formed some focal contacts and extensive close-contact zones, gave AF-actin TIRF images of relatively even intensity. Thin lamellar regions of the cytoplasm were found to contain concentrations of actin not significantly different from other close-contact regions of the cell. The major analytical problem of TIRF microscopy is separation of the effects of proximity to substrate, refractive index and fluorescent probe concentration on the local brightness of the TIRF image. From our results, it appears possible to use TIRF microscopy to measure the proximity of different components of substrate contact regions of cells.

Lanni, F., Waggoner, A. S., and *Taylor, D. L.*

The Journal of Cell Biology 100:1091-1102, 1985.

From the Center for Fluorescence Research in Biomedical Sciences and the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh.

LIGHT-SCATTERING CHANGES DURING CHEMOTACTIC STIMULATION OF HUMAN NEUTROPHILS: KINETICS FOLLOWED BY FLOW CYTOMETRY

The light-scattering properties of human neutrophils were compared on a cell-by-cell basis before and after stimulation with chemotactic peptide using flow cytometry. Between 20 and 180 sec after peptide addition, side (90°) scatter declined by up to 4% and forward scatter increased up to 6%. Between 3 and 15 min, side scatter increased up to 15% and forward scatter decreased up to 5%. Association of a fluorescence chemoattractant with neutrophils was most rapid during the initial phase of increasing forward and decreasing side scatter, and association saturated before the maximum increase in side scatter. Evidence is presented that the observed changes in scatter were not a consequence of chemoattractant-induced cell-cell adhesion or neutrophil degranulation. Rather, the early phases of light-scattering changes are interpreted to represent membrane ruffling by the stimulated neutrophil; the later phases polarization of the neutrophil morphology.

McNail, P. L., Kennedy, A. ., Waggoner, A. S., *Taylor, D. L.*, and Murphy, R. F.

Cytometry 6:7-12, 1985.

Other support: National Institutes of Health, National Science Foundation, and Health Research and Services Foundation.

From the Center for Fluorescence Research in Biomedical Sciences and the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh.

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CONDITIONS REQUIRED FOR EXPRESSION OF MEMBRANE IL 1 ON B CELLS

The authors have reported that macrophages bear a mitogenic activity for T cells in their plasma membrane that we have termed "membrane-interleukin 1". Membrane IL 1 was studied by using either paraformaldehyde-fixed macrophages or isolated macrophage membranes. The reason for ascribing this membrane activity to IL 1 was based on the observation that macrophage membranes stimulated proliferation of thymocytes and an IL 1-dependent T cell line but not IL 2-dependent T cells, and this activity was inhibited by a polyclonal goat anti-IL 1 antibody. Additional biochemical studies need to be done to unequivocally establish the interrelationships between the soluble IL 1 and the membrane form. Nevertheless, two important features of the membrane IL 1 were noteworthy: first, it was essential that Ia-bearing, fixed macrophages express it to activate antigen-specific T cell lines, and second, its expression could be dissociated from the secretion of IL 1. Because B cells are known to function as antigen-presenting cells, we investigated whether these cells express membrane IL 1.

Kurt-Jones, E. A., Kiely, J. M. and *Unanue, E. R.*

The Journal of Immunology 135(3):1548-50, 1985.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

IMMUNE COMPLEX EFFECT ON MURINE MACROPHAGES
I. IMMUNE COMPLEXES SUPPRESS INTERFERON-γ INDUCTION OF Ia EXPRESSION

We have studied the effects of immune complexes on the expression of macrophage surface proteins *in vitro*. Increased expression of the H-2 molecules I-A, I-E, and K on the macrophage membrane was induced by *in vitro* culture with crude lymphokine or interferon-γ. Expression of all three of the molecules was additional increased by stimulating the cultures with heat-killed *Listeria monocytogenes*. Addition of soluble immune complexes to the cultures did not have any effect on macrophage expression of these proteins. However, significant inhibition of lymphokine or interferon-γ induction of I-A, I-E, and H-2K was observed when macrophages were cultured on plates to which immune complexes had been bound. This inhibition was dose dependent, required an immunoglobulin (Ig) molecule with an intact Fc portion, did not require the presence of T cells, and occurred in the presence of indomethacin. Complexes containing IgG1, IgG2a, IgG2b, and IgE, but not IgM or IgA, antibodies mediated the inhibitory effect.

Virgin, H. W. IV, Wittenberg, G. F., and *Unanue, E. R.*

The Journal of Immunology 135(6):3735-3743, 1985.

Other support: National Institutes of Health and National Institute of General Medical Sciences.

From the Harvard Medical School, Department of Pathology, Boston.

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

IMMUNE COMPLEX EFFECTS ON MURINE MACROPHAGES. II. IMMUNE COMPLEX EFFECTS ON ACTIVATED MACROPHAGES CYTOTOXICITY, MEMBRANE IL-1, AND ANTIGEN PRESENTATION

We investigated the effects of immune complexes on macrophage functions *in vitro*. Immune complexes inhibit lymphokine induction of both I-A^k expression and cytotoxic activity by fetal calf serum elicited macrophages during long-term (7 days) culture. In addition, induction of antigen presentation was significantly inhibited by immune complexes. Expression of membrane interleukin 1 (IL-1, a membrane-bound form of the T cell mitogen required for antigen presentation by fixed cells) was minimally inhibited by immune complexes. Therefore, inhibition of antigen presentation was primarily due to effects on Ia expression rather than membrane IL-1 expression. The inhibitory effect of immune complexes was not found during short-term culture (4 to 48 hr) when activated macrophages (bearing high levels of Ia) from mice infected with *Listeria monocytogenes* were examined. Immune complexes maintained or even increased levels of both I-A^k and cytotoxicity in activated macrophages. The implications of these findings for immune complex modulation of the immune response are discussed.

Virgin, H. W. IV, Kurt-Jones, E. A., Wittenberg, G. F. and *Unanue, E. R.*

The Journal of Immunology 135(6):3744-3749, 1985.

Other support: National Institutes of Health; U. S. Public Health Service; National Institute of General Medical Sciences.

From Harvard Medical School, Department of Pathology, Boston, MA.

SUPPRESSION OF IMMUNE RESPONSE TO *Listeria monocytogenes*: MECHANISM(S) OF IMMUNE COMPLEX SUPPRESSION

We have investigated possible mechanisms underlying immune complex suppression of resistance to *Listeria monocytogenes*. Inhibition of resistance was found when immune complexes were formed *in vivo* in immune mice or in nonimmune mice adoptively transferred with specific antibody. Suppression was also found when nonimmune mice were injected with immune complexes preformed *in vitro*. We investigated the role of complement by decomplementing mice with cobra venom factor purified by high-pressure liquid chromatography. Complete depletion of serum C3 did not eliminate immune complex suppression of resistance to *L. monocytogenes*, suggesting that complement activation is not required for immune complex suppression. Infection-induced changes in the surface phenotype and functional properties of macrophages from normal and immune complex-suppressed mice were also investigated. Macrophage expression of both H-2K and Ia molecules increased during the response of normal mice to *L. monocytogenes*. However, these changes were not found in immune complex-suppressed mice. In contrast, membrane interleukin 1 expression was increased in macrophages from suppressed mice compared with macrophages from normal mice. Macrophages from *L. monocytogenes*-infected normal and immune complex-suppressed mice expressed cytotoxicity against tumor cells *in vitro*. We conclude that immune complexes do not inhibit resistance to *L. monocytogenes* by activation of complement or decreasing macrophage cytoxic activity. Rather, defects in Ia expression by macrophages from suppressed mice might be one component responsible for immune complex suppression of resistance of *L. monocytogenes*.

Virgin, H. W. IV, Wittenberg, G. F., Bancroft, G. J., and *Unanue, E. R.*

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

Infection and Immunity 50(2):343-353, 1985.

Other support: U.S. Public Health Service, National Institutes of Health, and National Institute of General Medical Sciences.

From the Department of Pathology, Harvard Medical School, Boston.

RELATIONSHIP OF MACROPHAGE Ia AND MEMBRANE IL 1 EXPRESSION TO ANTIGEN PRESENTATION

Membrane expression of Ia molecules by antigen-presenting cells is critical for the induction of T cell responses to foreign protein antigens. In addition, antigen-specific T cell proliferation has been thought to be dependent on interleukin 1 (IL 1) secretion by antigen-presenting cells. Recently, we have described a novel membrane-bound form of IL 1 that is required for the presentation of antigen by antigen-pulsed, fixed macrophages. Membrane IL 1 is a mitogenic protein found on the macrophage membrane that, like soluble IL 1, stimulates thymocytes and IL 1-dependent T cells but not IL 2-dependent T cell lines. This activity is inhibited by a polyclonal anti-IL 1 antibody. Membrane IL 1 is an integral membrane protein and does not represent soluble IL 1 nonspecifically bound or fixed to the macrophage membrane. Additional biochemical studies are required to define the possible interrelationships between soluble and membrane IL 1.

We have now developed a method for independently varying the levels of Ia and membrane IL 1 expression on macrophages in culture. By then fixing the cells, we are able to preserve constant levels of these molecules during the assay for antigen presentation. Using this system, we have demonstrated that quantitative variation in these two parameters was associated with changes in the magnitude of the T cell response.

Kurt-Jones, E. A., Virgin, H. W. IV, and *Unanue, E. R.*

The Journal of Immunology 135(6):3652-3654, 1985.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

ACUTE LUNG INJURY IN RAT CAUSED BY IMMUNOGLOBULIN A IMMUNE COMPLEXES

Mouse IgG and IgA, with reactivity to dinitrophenol conjugated to carrier protein, have been isolated from myeloma proteins by means of a variety of affinity techniques. The IgA was predominantly in the dimeric form. The *in vitro* and *in vivo* biological activities of IgA-containing immune complexes were assessed in the rat.

IgA-containing immune complexes were demonstrated, in a dose-dependent manner *in vitro*, to activate neutrophils and to generate O_2^-. In addition, these immune complexes showed evidence of complement activation *in vitro*, by the use of immunofixation techniques. When IgA was instilled into the airways of rats and antigen was injected intravenously, acute lung injury occurred, as reflected by increases in lung permeability and morphological changes consisting of blebbing of endothelial cells, intra-alveolar hemorrhage, and fibrin deposition. The lung changes were directly proportional to the amount of IgA instilled into the airways and failed to occur if intravenous injection of antigen was omitttedi Lung injury did not occur in animals that received an intravenous injection of antigen in the absence of an airway injection of

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IgA. Lung injury related to IgA-containing immune complexes was complement dependent but neutrophil independent. In companion studies with mouse IgG-containing immune complexes, acute lung injury also occurred and had morphological features similar to those associated with IgA-induced lung injury except that, in the case of IgG immune complex-induced damage, neutrophils were more evident. Acute lung injury induced by IgG-containing immune complexes, whether of mouse or rabbit origin, was complement and neutrophil dependent. The similarities and differences between IgG- and IgA-associated acute immune complex-induced injury of rat lung were reinforced by the use of morphometry techniques.

Studies with another monoclonal IgA antibody-containing, antigen-binding activity to phosphorylcholine also demonstrated the ability of IgA antibody to cause acute lung injury in the rat. Neither antibody alone nor antigen (phosphorylcholine linked to bovine serum albumin) alone produced evidence of lung injury.

These studies indicate for the first time that immune complexes containing IgA have lung-damaging properties and that the pathogenic mechanisms are different from those associated with IgG-associated immune complex-induced acute lung injury.

Johnson, K. J., Wilson, B. S., Till, G. O., and *Ward, P. A.*

Journal of Clinical Investigations 74:358-369, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, University of Michigan Medical School, Ann Arbor.

THE DNA SYNTHETIC RESPONSE OF NORMAL AND ABNORMAL HUMAN LYMPHOCYTES TO MEVALONIC ACID: THE ROLE OF GRANULOCYTES AS A HELPER POPULATION

In order to investigate the role of neutrophils in the DNA synthetic response of human peripheral blood lymphocytes to mevalonic acid, we obtained preparations of both cells each free of cross-contamination by the other. Purified lymphocytes respond poorly to mevalonic acid, but their response can be significantly enhanced by one-half their number of neutrophils. Preincubation of lymphocytes with neutrophils for 24 hr, even in the absence of mevalonic acid, further increases the lymphocyte response. We have been unable to demonstrate the production by granulocytes of either an intracellular or extracellular mevalonate-derived growth factor that in turn stimulates lymphocytes. Granulocytes preexposed to mevalonate do not acquire the ability to stimulate lymphocyte DNA synthesis in the absence of mevalonate. Our experiments suggest that neither neutrophil lysosomal enzymes nor reactive oxygen species generated by neutrophils are responsible for the help neutrophils provide. Normal E rosette-positive lymphocytes fail to respond to mevalonate, whereas E rosette-negative cells do. The mevalonate response of normal E rosette-negative cells is enhanced by the presence of granulocytes in contrast to B cell-chronic lymphocytic leukemia cells that synthesize DNA briskly in response to mevalonic acid in the absence of neutrophil help. These observations add to our knowledge of the relationship between mevalonate metabolism and the regulation of cellular DNA synthesis and mitosis.

Larson, R. A., Kluskens, L. E. and *Yachnin, S.*

Journal of Allergy and Clinical Immunology 74:280-291, 1984.

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

Other support: National Institute of Arthritis, Metabolism and Digestive Diseases and Nalco Cancer Research Fund.

From the Departments of Medicine and Pathology and the Committee on Immunology, The University of Chicago School of Medicine.

OXYGENATED CHOLESTEROLS SYNERGISTICALLY IMMOBILIZE ACYL CHAINS AND ENHANCE PROTEIN HELICAL STRUCTURE IN HUMAN ERYTHROCYTE MEMBRANES

Fourier transform infrared spectroscopy revealed that insertion of 20α-hydroxycholesterol into human erythrocyte membranes (10% of total membrane sterol) immobilized the lipid acyl chains to a degree equivalent to enriching total membrane cholesterol by 50%. Raman spectroscopy showed that the amount of acyl chain rotamers was not significantly altered by the presence of 20α-hydroxycholesterol, indicating that acyl chain immobilization was limited to an inhibition of lateral motion. The presence of 20α-hydroxycholesterol may synergistically enhance the acyl-chain-immobilizing behavior of membrane cholesterol. In addition, protein helical structure was not altered by 20α-hydroxycholesterol. The insertion of 7α-hydroxycholesterol into erythrocyte membranes resulted in an increase in protein helical structure which was comparable to that observed for erythrocyte membranes enriched with pure cholesterol by 50%. However, both acyl chain mobility and conformation were unchanged. These results suggest a synergistic behavior between oxysterols and cholesterol in modifying erythrocyte membrane packing.

Rooney, M. W., *Yachnin, S.*, Kucuk, O., Lis, L. J., and Kauffman, J. W.

Biochimica et Biophysica Acta 820:33-39, 1985.

Other support: Northwestern University Research and U.S. Public Health Service.

From the Biomedical Engineering Division, Northwestern University, Technological Institute, Evanston, IL; the Department of Medicine, the Pritzker School of Medicine, University of Chicago; the Department of Medicine, the Chicago Medical School; and the Department of Physics and the Liquid Crystal Institute, Kent State University, OH.

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VII. Metabolic Studies

EXPOSURE OF SMALL AIRWAYS TO CRISTOBALITE IN VITRO

In the studies reported here, tissue explants from different levels of rodent airways, from trachea to bronchioles as small as 200 microns in diameter, were exposed to nontoxic concentrations of cristobalite particles of a size less than 20 microns. The authors were interested in the effects of cristobalite, a known toxic and fibrinogenic particulate, upon mucin secretion and production of prostaglandins and leukotrienes by the exposed epithelial cells. They hypothesized that exposure to cristobalite could affect metabolism of arachidonic acid in these cells. Depending on the enzymatic pathways affected, this could lead to increased synthesis of products of the cyclooxygenase cascade that could inhibit mucin secretion (PGE) or stimulatory products of the lipoxygenase pathway (leukotrienes C and D). As was seen, the results of these studies suggest cristobalite has a differential effect on mucin secretion by explants of rodent airway tissue from different levels of the respiratory tree. As one progresses deeper into the intrapulmonary airways and into the small bronchioles, slight stimulstion of secretion changes to significant inhibition.

Adler, K. B. et al.

In: NATO ASI Series, Vol. G3, Beck, E. G. and Bignon, J. (eds.) *In Vitro Effects of Mineral Dust*. Berlin Heidelberg: Springer-Verlag, 1985.

Other support: National Institutes of Health.

From the Departments of Pathology, Civil Engineering and Physiology/Biophysics, University of Vermont, Burlington.

CYTOCHALASIN D-INDUCED INCREASE IN ACTIN SYNTHESIS AND CONTENT IN A VARIETY OF CELL TYPES

Treatment of a variety of mesenchymal cells (normal and transformed rat fibroblasts, bovine aortic endothelial cells, rabbit smooth muscle cells) exhibiting different cytoskeletal organizations and derived from several species with doses of cytochalasin D (CD, 2-6 μM for 20 h) sufficient to induce cytoskeletal rearrangement and altered cellular morphology results in an increase in the relative content and rate of synthesis of actin. These data extend our previous findings for HEp-2 cells to other cell types and provide further evidence for our hypothesis that the CD-induced cytoskeletal reorganization triggers stimulation of actin synthesis and the resulting increase in actin content.

Brett, J. G., Tannenbuam, J. and *Godman, G. C.*

Cell Biology International Reports 9(8):723-730, 1985.

Other support: National Science Foundation and National Institutes of Health.

From the the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

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MEMBRANE CYCLING AND MACROVACUOLATION UNDER THE INFLUENCE OF CYTOCHALASIN: KINETIC AND MORPHOMETRIC STUDIES

Fibroblasts exposed to higher doses of cytochalasin accumulate very big, discrete, endoplasmic vacuoles, the membrane of which is derived by internalization of plasmalemma. Morphometry confirms that the amount of surface interiorized is equal to the difference betwen the original cell surface area (before CD) and the reduced surface area measurable after CD-induced rounding. Correspondingly, there is a nearly two-fold increase in the activity of the ectoenzyme 5'-nucleotidase (a marker for plasma membrane) internally within the cytoplasm, after treatment with CD. Macrovacuolation increases cell volume by ~ 30%. Surface membrane is internalized as micropinocytotic vesicles at a rate measurable by the accumulation of horseradish peroxidase (HRP), a marker of fluid-phase pinocytosis. Uptake of HRP is shown to be enhanced at all times during exposure to CD and is balanced by accelerated exocytic recycling of membrane except during a phase (~ 4-8 hr) in which pinocytic uptake exceeds exocytosis. Vesicular membrane accumulated intracellularly in this period is retained in the endoplasm and by successive fusions forms vacuoles in close approximation to microfilament aggregates. Once established, this new macrovacuolar membrane compartment is in dynamic equilibrium with the cell surface and its membrane is cycled like the plasma membrane, in a mutual exchange of pinosomes between the several vacuoles and the cell surface. In drug-free medium, vacuole membrane apparently reverts to the surface by pinocytotic recycling, and the cells recover normal characteristics 4-6 hr after withdrawal of cytochalasin.

Brett, J. G. and *Godman, G. C.*

Tissue & Cell 16(3):325-335, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

PEROXISOMAL DEFECTS IN NEONATAL-ONSET AND X-LINKED ADRENOLEUKODYSTROPHIES

Accumulation of very long chain fatty acids in X-linked and neonatal forms of adrenoleukodystrophy (ALD) appears to be a consequence of deficient oxidation of very long chain fatty acids, a function that has been attributed to peroxisomes. Peroxisomes were readily identified in liver biopsies taken from a patient having the X-linked disorder. However, in liver biopsies from a patient having neonatal-onset ALD, hepatocellular peroxisomes were greatly reduced in size and number, and sedimentable catalase was markedly diminished. The presence of increased concentrations of serum pipecolic acid and the bile acid intermediate, trihydroxycoprostanic acid, in the neonatal ALD patient are associated with a generalized diminution of peroxisomal activities that was not observed in the patient with X-linked ALD.

Goldfischer, S. et al.

Science 227:67-70, 1985.

Other support: National Institutes of Health, National Science Foundation and Gail I. Zuckerman Foundation.

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

From the Departments of Pathology, Pediatrics, and Neurology and the Liver Research Center, Albert Einstein College of Medicine, The Bronx, NY; the Departments of Pathology and Pediatric Neurology, Children's Hospital of Michigan, and Wayne State University School of Medicine, Detroit; the Departments of Cell Biology and Medicine and Kaplan Cancer Center, New York University School of Medicine, New York; the John F. Kennedy Institute, the Johns Hopkins University School of Medicine, Baltimore, MD; and Rockefeller University, New York.

ULTRASTRUCTURAL AND CYTOCHEMICAL DEMONSTRATION OF PEROXISOMES IN CULTURED FIBROBLASTS FROM PATIENTS WITH PEROXISOMAL DEFICIENCY DISORDERS

The oxidation of very long chain fatty acids and synthesis of ether glycerolipids (plasmalogens) occurs mainly in peroxisomes. Zellweger's cerebrohepatorenal syndrome (CHRS) is a rare, inherited metabolic disease characterized by an apparent absence of peroxisomes, an accumulation of very long chain fatty acids, and a decrease of plasmalogens in tissues and cultured fibroblasts from these patients. As peroxisomes are ubiquitous in mammalian cells, we examined normal and CHRS-cultured fibroblasts for their presence, using an electron microscopic histochemical procedure for the subcellular localization of catalase, a peroxisomal marker enzyme. Small (0.08-0.20 μm) round or slightly oval peroxisomes were seen in both normal and CHRS fibroblasts. The number of peroxisomes was analyzed morphometrically and found to be significantly reduced in all CHRS cell lines. These results are discussed in relation to the underlying defect in peroxisomal function and biogenesis in this disease.

Arias, J. A., Moser, A. B., and *Goldfischer, S. L.*

The Journal of Cell Biology 100:1789-1792, 1985.

Other support: National Institutes of Health.

From the Albert Einstein College of Medicine, The Bronx, NY, and The Johns Hopkins School of Medicine, Baltimore, MD.

PROCESSING OF ANGIOTENSIN AND OTHER PEPTIDES BY THE LUNGS

The pulmonary vascular bed can be considered as a sluice gate for controlling the quality of biologically active peptides allowed to enter the systemic circulation. The remarkable feature of the lung is the selectivity of the processing, especially when one considers that most peptides are indiscriminately degraded by blood enzymes, tissue homogenates, and many enzymes. One of the most critical determinants of the selectivity of metabolism of peptides is the location of their inactivating enzymes. Thus it is the cell biology that to a great extent determines the access of peptides to their metabolizing enzymes. The present chapter is devoted to the processing of polypeptide hormones by the lungs. Bradykinin and angiotensin (ANG) are the most studied of the physiologically important peptide hormones processed by the lungs. In the discussion section of this paper, it is noted that the biological activities of the kinins and ANG II disappear, whereas the activity of ANG I is enhanced during circulation through various vascular beds. To a large degree the changes in activities of the vasoactive polypeptides are independent of enzymes and cellular elements of blood but are closely related to peptidase activities of the vascular bed itself. Over the past several years, aspects of the metabolism of kinins and ANG I by pulmonary endothelium have been defined. The

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focus has been on a cell not previously known to exist until the advent of electron microscopy. Endothelial cells selectively process a series of vasoactive substances, including ANG I and bradykinin. Stidues of the pulmonary processing of angiotensins and related peptides clearly show that the critical, initial reactions occur at the cell surface — this is true of metabolic enzymes, receptors, and the release of products. A challenge for the future is to begin to chart the geography of the pulmonary endothelial cell surface.

Ryan, U. S.

In: Fishman, A. P. and Fisher, A. B. (eds.): *Handbook of Physiology - The Respiratory System I*, Bethesda, MD: The American Physiological Society, 1985, Chap. 10, pp. 351-364.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

THE PULMONARY ENDOTHELIAL SURFACE

The understanding of endothelial metabolic properties has increased dramatically in recent years. Endothelial cells (ECs) possess hormones, drugs, and many blood-borne substances by means of enzymes and transport processes. In turn, some hormones, blood cells, and cellular products interact with ECs via specific receptors on the luminal surface. Functional complexity is exemplified by the metabolism of the adenine nucleotides. ATP, ADP, and AMP are metabolized by enzymes of the endothelial surface to release adenosine, which may be immediately taken up into endothelium and reincorporated intracellularly into nucleotides. Equally complex is the metabolism of the kinins and angiotensins by ECs. Bradykinin is inactivated whereas angiotensin I is converted to angiotensin II. Bradykinin not thus degraded can act on endothelial receptors and stimulate the release of prostacyclin (PGI_2). Thus, bradykinin can amplify the release of another vasodilator, PGI_2, and can stimulate the release of a powerful antiaggregatory agent (PGI_2). Many of these complex metabolic reactions occur at the endothelial surface, a structure that is itself complex. ECs possess endothelial projections and caveolae as well as a fuzzy coat, or glycocalyx. Functions of the endothelial glycocalyx are not well understood, but the glycocalyx cannot be visualized; it may act as a molecular sieve and provide a substratum for the initiation and progression of immunologic reactions.

Ryan, U. S., Ryan, J. W. and Crutchley, D. J.

Federation Proceedings 44:0013-0019, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL, and the Research Division, Miami Heart Institute, Miami Beach, FL.

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PULMONARY ENDOTHELIUM AND PROCESSING OF PLASMA SOLUTES: STRUCTURE AND FUNCTION

While the lungs often are considered exclusively in terms of gas exchange, they have additional functions not necessarily related to such exchange. One such activity is the ability to process selectively hormones, hormone precursors, and other excitatory substances as they pass through the lungs via the bloodstream. As the lungs convert venous blood to arterial blood, they also regulate the entry of hormonal substances into the systemic arterial circulation. Through this selective processing, products of specific reactions of the lungs can influence specific actions of tissues and organs at a distance. The first level of compartmentation in the lungs is the separation of the blood supply from the air supply. Quite clearly, circulating substances are unlikely to have access to enzymes beyond the first cellular layer lining the vessels — the endothelium. The present chapter describes functional and structural aspects of the interaction of solutes and colloids of plasma with pulmonary endothelial cells. First the basic architecture and environment of the lungs are considered. The structure and situation of the lungs within the circulatory system which make them well suited for gas exchange may well explain how the lungs are so efficient in processing some hormones. The next level of compartmentation is within the pulmonary circulation and is governed by the flow characteristics and relative surface areas of the vessels. Figures are given in this section which emphasize the overwhelming significance of endothelial cells of the pulmonary microvasculation in providing a surface for interaction with blood-borne substrates. In following sections of this paper, Immunocytochemistry, Endothelial Cell Culture and Surface Specializations (Endothelial Projections, Caveolae, and Glycocalyx), are discussed. It will be important for future studies to examine to what extent modulation of endothelial surface structure — projections, caveolae, glycocalyx, enzyme receptors, and transport molecules — affect the overall functioning of endothelium as a tissue and the overall functioning of the lungs in maintaining the quality of the internal milieu.

Ryan, U. S. and Frøkjaer-Jensen, J.

In: Said, S. I. (ed.): *The Pulmonary Circulation and Acute Lung Injury*. Mount Kisco, NY: Futura Publishing Co., Inc., 1985, pp. 37-60.

Other support: National Institutes of Health.

From the University of Miami School of Medicine, Miami, FL.

EVIDENCE FOR A ROLE OF HYDROXYL RADICAL IN IMMUNE-COMPLEX-INDUCED VASCULITIS

Previously it was shown that tissue injury occurring in acute immune-complex-induced vasculitis, which is complement- and neutrophil-dependent, is significantly attenuated by the presence of catalase, suggesting the pathogenic role of H_2O_2 generated from activated neutrophils. We now show that significant protection is also afforded by pretreatment of animals with apolactoferrin, a naturally occurring chelator of iron. Iron-saturated lactoferrin is devoid of protective effects. Deferoxamine mesylate, a synthetic iron chelator, also has protective effects. Infusion of ionic iron, especially Fe(III), potentiates the tissue injury. Significant protection from tissue injury is also produced by treatment of rats with dimethyl sulfoxide, a potent hydroxyl radical scavenger. Morphologically, animals treated with these protective interventions show the influx of neutrophils into sites of immune complex deposition, but there is markedly attenuated edema, little or no hemorrhage, and little evidence of

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endothelial cell injury, in contrast to the findings in nonprotected animals. These data support the suggestion that immune-complex-induced injury may be linked to generation of H_2O_2 from activated neutrophils and the subsequent conversion of peroxide to the hydroxyl radical.

Fligiel, S. E. G., *Ward, P. A.*, Johnson, K. J., and Till, G. O.

American Journal of Pathology 115:375-382, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, the University of Michigan Medical School, Ann Arbor.

EVIDENCE FOR THE ROLE OF OXYGEN RADICALS IN ACUTE NEPHROTOXIC NEPHRITIS

Acute glomerular injury in the rat has been induced by the intrarenal, intraarterial infusion of sheep antibody to glomerular basement membrane anti-GBM (antiglomerular basement membrane). The antiglomerular basement membrane antibody has been verified to be of the variety that is complement and neutrophil dependent for the induction of acute proteinuria, which peaks during the first 24 hours. Following injection of the antibody, acute, intense, glomerular injury resulted, with the denuding of glomerular vascular basement membrane associated with extensive damage or destruction of glomerular endothelial cells and fusion of epithelial cell foot processes. Treatment of animals with catalase produced, in a dose-dependent manner, as much as 75% protection against glomerular injury, as assessed by reduction in the proteinuria. Treatment of animals with superoxide dismutase caused a small reduction in the degree of glomerular injury, again assessed by a reduction in proteinuria. However, this protective effect of superoxide dismutase was not found to be statistically significant. The hydroxyl radical scavenger, dimethyl sulfoxide, which has been shown to protect against endothelial cell injury following systemic activation of complement, was not protective in the anti-GBM model. Morphologically, glomeruli from catalase-protected rats showed numerous neutrophils but little or no evidence of injury of either glomerular endothelial or epithelial cells. These data suggest that acute glomerular injury produced by antiglomerular basement membrane is related to H_2O_2 production from activated neutrophils.

Rehan, A., Johnson, K. J., Wiggins, R. C., Kunkel, R. G., and *Ward, P. A.*

Laboratory Investigation 51(4):396-402, 1984.

Other support: National Institutes of Health.

From the Departments of Internal Medicine and Pathology, the University of Michigan Medical School, Ann Arbor.

RAT NEUTROPHIL ACTIVATION AND EFFECT OF LYPOXYGENASE AND CYCLOOXYGENASE INHIBITORS

Activation (defined as lysosomal enzyme secretion and generation of O_2^- of rat neutrophils has been measured with the use of varying doses of soluble stimuli (phorbol myristate acetate (PMA); calcium ionophore A23187; and N-formyl-methionyl-leucyl-phenyl-alanine (FMLP) and particulate agents (immune complexes

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and zymosan particles). With either the calcium ionophore or the chemotactic peptide (FMLP), substantial enzyme release occurred, but the amount of O_2^- produced was very small. Cytochalasin B greatly enhanced the enzyme release response to the chemotactic peptide but had little effect on neutrophil responses to other soluble stimuli. The cell response to PMA resulted in the greatest production of O_2^- with significant enzyme secretion. When cell stimulation with insoluble stimuli (immune complexes or zymosan particles) was studied, significant amounts of enzyme release occurred in parallel with the generation of substantial amounts of O_2^-. The presence of cytochalasin B enhanced the cell responses to immune complexes but had an inhibitory effect on zymosan-induced responses. As expected, the amount of lysozyme secreted by stimulated rat neutrophils tended to exceed the amount of β-glucuronidase released from the same cells.

Neutrophil responses were investigated in the presence of drugs that were demonstrated in the rat neutrophil to inhibit either the lipoxygenase of the cyclooxygenase pathways. Inhibitors of the cyclooxygenase pathway (indomethacin, piroxicam, ibuprofen, BW755C), with few exceptions, consistently enhanced the enzyme secretion response, while effects on O_2^- generation were less clear-cut but tended to be predominantly inhibitory. Drugs with inhibitory effects on the lipoxygenase pathway (nordihydroguaiaretic acid and nafazatrom) had significant inhibitory effects on both enzyme secretion as well as generation of O_2^-. These data suggest that activation responses (enzyme secretion and O_2^- generation) of rat neutrophils may be dissociated (*i.e.*, one not always accompanying the other). Further, it appears that neutrophil activation, as defined by enzyme secretion, is enhanced by products of the lipoxygenase pathway and suppressed by products of the cyclooxygenase pathway. Generation of O_2^- is not affected in such a clear-cut manner. Taken together, the data suggest that enzyme release and O_2^- production by activated rat neutrophils may be under separate control.

Ward, P. A., Sulavik, M. C., and Johmson, K. J.

American Journal of Pathology 116:223-233, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, the University of Michigan Medical School, Ann Arbor.

DIFFERENTIATION OF A HUMAN LEUKEMIA CELL LINE AND EXPRESSION OF COLLAGENASE INHIBITOR

A human collagenase inhibitor (CI) of M_r 29,500 has been extensively characterized in skin fibroblasts and identified in a variety of connective tissues. Because human alveolar macrophages synthesize and secrete both a collagenase and CI that are immunologically and functionally identical to their counterparts in fibroblasts, we studied the production of such proteins by an immature human cell line (HL60) that can be induced to differentiate along monocytic or granulocytic pathways. The cells failed to synthesize collagenase under any culture condition tested. However, upon exposure to 1,25-dihydroxyvitamin D_3 or phorbol esters (PMA), both of which promote monocytic differentiation of HL60, these cells synthesized and released CI in a dose-dependent manner. Furthermore, the extent of CI expression was paralleled by the acquisition by such cells of the monocytic marker 63D3, indicating that inhibitor production and differentiation are closely correlated. This CI was immunologically and functionally

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identical to that produced by human macrophages and human skin fibroblasts. The quantity of CI synthesized by PMA-stimulated cells was 3- to 5-fold greater than produced by human alveolar macrophages, ≈ 1 μg per 10^6 cells per day. In contrast, undifferentiated HL60 cells produced little or no detectable CI (≤ 10-20 ng per 10^6 cells per day). Interestingly, when HL60 cells were stimulated to undergo granulocytic differentiation by dimethyl sulfoxide or retinoic acid, they also produced the "monocytic" CI.

Shavit, Z. B., *Welgus, H. G. et al.*

Proceedings of the National Academy of Sciences of the United States of America 82:5380-5384, 1985.

Other support: National Institutes of Health.

From the Division of Cell Biology, Washington University School of Medicine; Division of Dermatology, Department of Medicine, The Jewish Hospital at Washington University Medical Center, St. Louis; and Division of Dermatology, Department of Medicine, VA Medical Center/University of Tennessee Center for the Health Sciences, Memphis.

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FAMILIAL AGGREGATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: USE OF THE LOGLINEAR MODEL TO ANALYZE INTERMEDIATE ENVIRONMENTAL AND GENETIC RISK FACTORS

To examine the contribution of environmental and genetic risk factors to familial aggregation in chronic obstructive pulmonary disease (COPD), 325 first-degree (1d) relatives and 56 spouses of 150 COPD patients were compared with 222 1d relatives and 49 spouses of 107 nonpulmonary patient controls for the prevalence of two clinical outcomes: 1) airways obstruction (AO; 1-sec forced expiratory volume ≤ 68% of forced vital capacity) and 2) chronic bronchitis (CB; cough and sputum for 3+ months per year for 2+ years). The loglinear model was used to study direct and indirect (*i.e.*, those mediated by other risk factors) components of familial aggregation. Three risk factors were found to be independently associated with CB and/or AO: α_1-antitrypsin deficiency (PiZ allele), personal cigarette smoking, and parental cigarette smoking. Because 1d relatives of COPD patients were more likely to have a PiZ allele, be heavy smokers (1+ packs per day), and be exposed to parental smoking than 1d relatives of controls, these three factors also constituted indirect components of familial aggregation. However, after controlling for the three factors, 1d relatives of COPD patients were more likely to have AO and CB than 1d relatives of controls (direct component). This direct component might have a genetic basis because no such association was found when spouses instead of 1d relatives were compared. Thus, both shared environmental factors (personal and passive smoking) and shared genetic factors (α_1-antitrypsin and a possible direct genetic component) contribute to familial aggregation in COPD. The loglinear model provides a useful tool for analyzing familial aggregation in diseases of multifactorial etiology.

Khoury, M. J., Beaty, T. H., Tockman, M. S., Self, S. G., and *Cohen, B. H.*

Genetic Epidemiology 2:155-166, 1985.

Other support: Lebanese National Council for Scientific Research and National Institutes of Health.

From the Departments of Epidemiology, Biostatistics and Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, Baltimore.

HEALTH RELATED PSYCHOSOCIAL CORRELATES OF NEUROTICISM: A STUDY OF ADULT MALE TWINS IN FINLAND

Some health related psychosocial correlates of the Eysenck neuroticism scale were examined in a questionnaire study of 5001 monozygotic (MZ) and 3455 dizygotic (DZ) male twin pairs representing the adult male twin population in Finland. In analyses of the individuals, 34% of the variance in neuroticism was associated with psychological variables (stress of daily activities, life satisfaction, quality of sleep, and extroversion—the explanatory rate of this variable set was 30%), psychotropic drugs (5%), alcohol use (4%), and smoking (2%). Neuroticism was also associated with social, life change, and medical variables. In pairwise analyses, the heritability estimate (h^2) was 0.54 for pairs living together and 0.39 for pairs living apart. It seems that heritability

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estimates are confounded by the closer intrapair relationship between members of MZ than DZ pairs. In pairwise analyses, 23% of the intrapair difference of neuroticism in MZ pairs was associated with intrapair differences in the aforementioned variables. The following explananatory rates were found: psychological variables, 21%; psychotropic drugs, 2%; alcohol use, 2%; and smoking, 1%. Neuroticism of pairs discordant for background variables showed similar intrapair differences as between individuals in the following variables: service *vs.* farming work, use of alcohol, use of antacids, hypertension, heavy physical work, quality of sleep, changes of workplace for negative reasons, smoking, and use of tranquillizers. It appears that in Finland, environmental factors explain at least 61% of the variability in neuroticism, and that factors determining neuroticism are also associated with health related behavior such as smoking, use of alcohol and psychotropic drugs.

Koskenvuo, M., Langinvainio, H., Kaprio, J., and Sarna, S.

Acta Geneticae Medicae et Gemellologiae 33:307-320, 1984.

From the Department of Public Health Science, University of Helsinki, Helsinki, Finland.

FINNISH TWINS REARED APART II. VALIDATION OF ZYGOSITY, ENVIRONMENTAL DISSIMILARITY AND WEIGHT AND HEIGHT

Within the Finnish Twin Cohort of like-sexed adult twin pairs, a subgroup of pairs separated at an early age has been identified. In 165 pairs, both cotwins responded to questionaires in 1975 and 1979. An environmental dissimilarity score was formed which consists of items on whether the twins had lived after separation in the same community, attended the same school, were in the same grade at school, how often they met, how often they met common friends and relatives, and whether they attended the same clubs or not, *etc.* To validate the zygosity diagnosis obtained by questionnaire in 1975, those pairs whose zygosity was unknown and those with the least contact after separation were contacted for blood sampling (11 bloodgroups). Of 15 pairs with no zygosity diagnosis, 10 responded (1 no address, 2 abroad, 2 refused). Six pairs were classified MZ and 4 DZ. In 12 MZ and 8 DZ pairs undergoing bloodgroup determination, the classification of only one pair changed from DZ to MZ. The following intraclass correlations for height and weight were found:

Age at	No. Cases		Weight		Height	
separation	MZ	DZ	MZ	DZ	MZ	DZ
0-5	18	61	0.88	0.31	0.88	0.70
0-10	30	95	0.87	0.36	0.92	0.70

Langinvainio, H., *Koskenvuo, M.*, Kaprio, J., and Sistonen, P.

Acta Geneticae Medicae et Gemellologiae 33:251-258, 1984.

Other support: Yrjo Jahnsson Foundation.

From the Department of Public Health Science, University of Helsinki, and Finnish Red Cross Blood Transfusion Service, Helsinki, Finland.

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FINNISH TWINS REARED APART III. PERSONALITY FACTORS

This study is based on data from 165 adult twin pairs separated at 10 years of age or less. Information on personality factors: extraversion (E) and neuroticism (N) (EPI scale short form), life satisfaction (LS) (Allardt) and stress of daily activities (SDA) was obtained as part of the questionnaire study carried out in the entire Finnish Twin Cohort in 1975. Later in 1979 a questionnaire sent to the twins reared apart yielded a scale (range 7-30 points) measuring the environmental dissimilarities after separation (reliability 0.83). The effect of separation on personality factors by analysis of variance of individual data was studied. The overall explanatory rates were low (2.1 - 4.4%). The definitive study group was formed by selecting those pairs with a dissimilarity score greater than 15. The following intraclass correlations were obtained:

Age at separation	No.of cases		E		N		LS		SDA	
	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
0.5	18	61	0.40	0.17	0.34	0.07	0.22	0.18	0.04	0.00
0-10	30	95	0.38	0.12	0.25	0.11	0.40	0.19	0.06	0.02

Langinvainio, H., Kaprio, J., *Koskenvuo, M.*, and Lonnqvist, J.

Acta Geneticae Medicae et Gemellologiae 33:259-264, 1984.

Other support: Yrjo Jahnsson Foundation

From the Departments of Public Health Science and Psychiatry, University of Helsinki, Finland.

PSYCHIATRIC HOSPITALIZATION IN TWINS

Hospitalization rates of monozygotic (MZ) and dizygotic (DZ) twin pairs in Finland were compared for schizophrenia, neuroses, and alcoholism. Record-linkage of hospital records and death certificates for the years 1972-1979 was carried out for persons in the Finnish Twin Cohort (16,649 like-sexed twin pairs). The ratio of the number of observed *vs.* that of expected concordant pairs and the ratio of concordance rates between MZ and DZ pairs were greater among males than females, and greater among young (40 years old or less) than among older pairs. The highest difference was found in schizophrenia and the lowest in neuroses. Pairwise concordance rates for schizophrenia (11.0% for MZ and 1.8% for DZ) seem to indicate great environmental influence (high proportion of discordant pairs) with apparent genetic liability (6.1-fold ratio in concordance between MA and DZ pairs). In neurotic disorders, the difference of pairwise concordance rates between MZ and DZ pairs (0.0% vs. 4.0%) was quite low, not strongly supporting a genetic hypothesis. Of the MZ pairs concordant for psychiatric hospitalization, 47% had lived together for their whole life time; of those discordant, 16% lived together. The corresponding figures for DZ pairs were 18% and 15%. The effect of intrapair relationships in disease-concordant pairs should be taken into account when evaluating the effect of genetic and environmental factors in psychiatric disorders.

Koskenvuo, M., Langinvainio, H., Kapirio, J., Lonnqvist, J., and Tienari, P.

Acta Geneticae Medicae et Gemellologiae 33:321-332, 1984.

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

From the Department of Public Health Science and Department of Psychiatry, University of Helsinki, and Department of Psychiatry, University of Oulu, Oulu, Finland.

SNORING AS A RISK FACTOR FOR HYPERTENSION AND ANGINA PECTORIS

The association of snoring with hypertension and ischaemic heart disease (IHD) was tested by postal questionnaire in a population of 3847 men and 3664 women aged 40-69 years. Hypertension associated highly significantly with snoring, the relative risk (RR) of hypertension between habitual snorers and never snorers being 1.94 in men and 3.19 in women. This association was also found when adjusting for body-mass index. A significant association between angina pectoris and habitual snoring was observed in men (RR = 2.22). In women the relative risk was not significant. An association between habitual snoring and angina pectoris in men was also found after adjusting for hypertension and body-mass index (RR = 2.01, $p<0.01$). The relative risks for myocardial infarction and hospital admission for IHD for habitual snorers were non-significant.

Koskenvuo, M. et al.

The Lancet (I)893-896, 1985.

Other support: The Medical Research Council, Academy of Finland, and the Paavo Nurmi Foundation.

From the Departments of Public Health Science and Neurology, University of Helsinki, Finland.

CANCER STUDIES IN TWINS AND FAMILIES OF TWINS

The study of concordance for cancer in twin pairs can be used to examine the role of heritable factors in cancer. The Finnish Twin Cohort — all like-sexed adult twin pairs born before 1958 in Finland with both co-twins alive in 1967 (n = 17,357 pairs) — was linked to the Finnish Cancer registry data to yield incident cases of cancer up to 1981. Zygosity was determined by the validated questionnaire method in 1975.

A total of 1,112 cases of cancer was found in the twin series up to 1981 among 1,068 twins. Multiple primary cancer was found among 40 persons (3.8%). There were 233 MZ pairs discordant for cancer and 18 pairs in which both members had cancer (probandwise concordance rate = 13.4%). In the DZ pairs there were 478 ciscordant pairs and 34 concordant pairs (probandwise concordance rate 12.5%). When cases of low malignancy were excluded (basal cell carcinoma, *in situ* uterine cervix carcinoma, urinary bladder papilloma, polycythemia vera and myelofibrosis) the figures for MZ pairs were 12 concordant and 202 discordant (rate 10.6%), and for DZ pairs 23 concordant and 413 discordant (rate 10.0%).

The analysis of concordance indicates that overall hereditary factors do not contribute much to the incidence of cancer. It is in accordance with the epidemiologic evidence for the primary role of environmental factors in the etiology of most cancers.

Kaprio, J., *Koskenvuo, M.*, Teppo, L., Langinvainio, K., Pukkala, E., Rita, H., and Sarna, S.

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Source: https://www.industrydocuments.ucsf.edu/docs/tqxk0000

In: *Familial Cancer*. 1st International Research Conference, Basel 1985, pp. 192–194 (Karger, Basel, 1985).

Other support: Sigrid Juselius Foundation.

From the Finnish Twin Cohort Study, Department of Public Health Science, University of Helsinki, and Finnish Cancer Registry, Helsinki, Finland.

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